

Expression patterns of a novel *AtCHX* gene family highlight potential roles in osmotic adjustment and K⁺ homeostasis in pollen development

Heven Sze, Senthilkumar Padmanaban, Kevin W. Bock & Xiyang Li, University of Maryland, College Park, MD 20742-5815, USA

Françoise Cellier & Genevieve Conéjéro, INRA/CNRS/AgroM/UMII, Montpellier, France

David Honys, Institute of Experimental Botany AS CR, Praha, Czech Republic

Ning-Hui Cheng & Kendal D. Hirschi, Baylor College of Medicine, Houston, TX, USA

David Twell, University of Leicester, Leicester LE1 7RH, England

John M. Ward, University of Minnesota, St. Paul, MN, USA

A combined bioinformatic and experimental approach is being used to uncover the functions of a novel family of *CHX* (cation/H⁺ exchanger) genes in plants using *Arabidopsis thaliana* as a model. The predicted protein (85-95 kDa) of 28 *AtCHX* genes after revision consists of an amino-terminal domain with 10-12 transmembrane spans (~440 residues), and a hydrophilic domain of ~360 residues at the carboxyl end which is proposed to have regulatory roles. The hydrophobic, but not the hydrophilic, domain of plant CHX is remarkably similar to monovalent cation/proton antiporter-2 (CPA2) proteins, especially yeast KHA1 and Synechocystis NhaS4. Reports of characterized fungal and prokaryotic CPA2 indicate they have various transport modes, including K⁺/H⁺ (KHA1), Na⁺/H⁺-K⁺ (GerN) antiport, and ligand-gated ion channel (KefC). The expression pattern of *AtCHX* genes was determined by reverse-transcription polymerase-chain-reaction, promoter-driven GUS expression in transgenic plants, and Affymetrix ATH1 Genome Arrays. Results show that 18 genes are specifically or preferentially expressed in the male gametophyte and 6 genes are highly expressed in sporophytic tissues. Microarray data revealed that several *AtCHX* genes were developmentally-regulated during microgametogenesis. An exciting idea is that CHX proteins allow osmotic adjustment and K⁺ homeostasis as mature pollen dessicates and then rehydrates at germination. The multiplicity of *CHX*-like genes is conserved in higher plants, but is not found in animals. Only seventeen genes, *OsCHX01-OsCHX17*, were identified in *Oryza sativa* ssp. japonica, suggesting further diversification in Arabidopsis. These results reveal a novel *CHX* gene family in flowering plants with potential functions in pollen development, germination, and tube growth.